IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Thomas Stormann et al.

Serial No.: 09/679,664

Filed: October 3, 2000

For: G-PROTEIN FUSION RECEPTORS AND CHIMERIC GABAb RECEPTORS

Examiner: Robert Landsman

Group Art Unit: 1647

Attorney Docket No.: 7394US

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DECLARATION OF LAURA L. STORJOHANN

Commissioner for Patents
P.O. Box 1450
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Sir:

I, Laura L. Storjohann, declare that:

1. I am over twenty-one years of age and competent to give sworn testimony.

2. I am a named co-inventor in United States Patent Application Serial No. 10/679,664, entitled "G-Protein Fusion Receptors and Chimeric $GABA_b$ Receptors," filed on October 3, 2000. I am a molecular biologist in the drug discovery department at NPS Pharmaceuticals with more than 14 years experience in both academic research and in the pharmaceutical industry. I have been actively involved in research in the areas of mGluR constructs, G-protein fusion constructs, and mGluR/CaR chimeric

constructs from 1996 to present. I am a named author in eight publications and abstracts relating to glutamate receptors and/or mGluR/CaR chimeras.

3. The purpose of creating functional chimeric fusion receptors is for their utility in high throughput screening of compound libraries in order to identify compounds that interact with the native receptor. Compounds identified in these screens are not specific to the chimeric fusion receptor; rather they will act on the native receptor as well. The unique signaling properties of the chimeric fusion receptors simply allow a more efficient method for identifying these compounds as leads for drug candidates.

A second utility is the ability to target and/or identify the specific domain in which a compound acts. The extracellular domain of Class 3 (formerly known as Family C) GPCRs, including metabotropic glutamate receptors, $GABA_B$ receptors, calcium receptor and others, contains the binding site for the endogenous agonists. The transmembrane domain (TMD) of these receptors, however, contains a second ligand binding site that may be important for drug discovery, though is not known to bind any endogenous ligand. NPS and other drug companies have identified drug candidate leads that bind in the TMD and modulate the activity of the receptors (for a recent review see May & Christopoulos *Current Opinion in Pharmacology* 2003). These are known as allosteric modulators of GPCRs. In fact, NPS discovered and Amgen developed Sensipar®, an approved drug that acts as a positive allosteric modulator of the calcium receptor and binds the calcium receptor within the transmembrane domain. So these chimeric fusion receptors are valuable tools to find transmembrane-acting compounds. For example, using a construct comprised of the extracellular domain of the calcium receptor with the transmembrane and intracellular domains of mGluR2, then fused to a promiscuous G protein allows one to screen compound libraries on the FLIPR. These "hits" can then be screened against the calcium receptor to discard compounds that act on the calcium receptor. The remaining hits are thus targeted to the TMD of mGluR2.

Class 3 GPCRs, when heterologously expressed, are either able to signal through the phospholipase C (PLC) pathway or through the cyclic AMP second messenger system. For technical reasons, it has historically been much simpler to

screen compounds on PLC-coupled receptors. The design of chimeric receptors fused to promiscuous G proteins enabled efficient high throughput screening of normally cyclic AMP-coupled receptors, as described further below.

Group I mGluRs, comprised of mGluR1 and mGluR5, along with the calcium receptor (CaR) interact via Gαq to activate the phospholipase C signal transduction pathway. Activation of these receptors by, for example, their endogenous ligand causes a conformational change in the Gα subunit of the heterotrimeric G protein and catalyzes the exchange of GDP for GTP on the Gα subunit. The heterotrimeric G protein then dissociates into separate β/γ and α-GTP (active) subunits. The active Gα subunit activates phospholipase C (PLC) which in turn catalyzes the reaction that generates inositol trisphosphate (IP3) and leads to calcium release from IP3-regulated Ca^{2+} pools located in the endoplasmic reticulum. This intracellular calcium mobilization is readily measured in high throughput format utilizing the FLIPR device (Molecular Devices Corporation).

In contrast, the Group II and Group III mGluRs, which couple predominantly through the Gαi/o family of G proteins to inhibit adenylyl cyclase, have presented challenges to the development of robust and efficient high throughput screening (HTS) assays. To address this, we have employed chimeric receptors and promiscuous G proteins that together are able to force Group II and Group III mGluR (and other Gαi/o-coupled GPCRs) chimeras to signal through the phospholipase C so that the resulting intracellular calcium mobilization could be measured in high throughput format utilizing the FLIPR device.

One type of chimera that was especially useful in setting up these high throughput screens for Group II and III mGluRs was swapping the intracellular domain of the mGluR with that of the calcium receptor. This increased the ability of the receptor to interact with promiscuous Gα proteins, such as Gαqi5, Gαqo5 or Gα15. These promiscuous Gα proteins are able to interact with receptors that normally interact with Gαi/o family of G proteins, such as mGluR2, and their activation stimulates the phospholipase C pathway to mobilize calcium from intracellular stores. In effect, the use of the intracellular domain of the calcium receptor increased the signal to noise ratio in these screening assays.

We also have found that fusing the promiscuous Gα protein to the chimeric receptor had many advantages for assay development. First, the receptor-G protein stoichiometry remains constant. Second, it allows for transfection of a single construct, rather than two. And finally, in co-transfected cells we've seen Gα protein expression decrease over time, which does not occur in these fusions. Thus, cell lines expressing these chimeric fusion constructs remain stable and efficient tools for compound screening. Based on my experience and education, I believe that a person having skill in the art of molecular biology, like myself, would know how to use the methods and receptors that are claimed in the present application by reading the description provided in the Specification of the application.

5. Claim 1 of the present patent application recites that an intracellular domain joined to the carboxy terminus of the transmembrane domain comprises all or a portion of an intracellular amino acid sequence at least 75% identical to either an intracellular CaR amino acid sequence, an intracellular mGluR amino acid sequence, or an intracellular $GABA_B$ receptor amino acid sequence, provided that said portion is at least 10 amino acids. Such functional receptors having an intracellular domain sequence of 10 amino acids in length can be constructed. In fact, I and others at NPS Pharmaceuticals have constructed intracellular domains having 10 amino acid residues of CaR and MGluR2, which produced functional intracellular tails.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the U.S. code and that such willful false statements may jeopardize the validity of the patent.

Date: 07/25/2005 Laura L. Storjohann

Laura L. Storjohann